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LIFE CYCLES OF EUROPEAN PTERIDOPHYTES

PETER R. BELL
Department of Botany and Microbiology
University College, London, WC1E 6 BT. England

Summary.

Although the need for water for fertilization restricts the distribution of pteridophytes, some are able to persist in remarkably dry situations. Fertility in the sexual cycle may be further limited by several factors, amongst them errors of fertilization, competition between embryos, self-incompatibility, accumulated sporophytic lethals (genetic load) and, in some species, possible physiological interference between the expression of maleness and femaleness in the same gametophyte.

Aberrant life-cycles are common in the European ferns. All, so far as known, are dependent on the formation of restitution nuclei, leading to sporangia with 8 spore mother cells. Embryos are formed apogamously. A few life cycles involve apospory and apogamy (and perhaps parthenogenesis), but these are maintained solely in cultivation.

Heterosporous cycles are also found in European pteridophytes. In *Marsilea* and *Pilularia* the formation of viable megaspores involves the regular resorption of three spores in each tetrad, similar to the situation in the seed plants. In *Selaginella* megasporogenesis is less regular, involving loss of both megaspore mother cells and meiotic products. The formation of the megaspores in *Isoetes* appears to follow simply from the many fewer mother cells in the megasporangium. Both *Selaginella* and *Isoetes* seem to represent attempts at heterosporous cycles of limited evolutionary success.

Key words: fertility, incompatibility, apogamy, heterospory.

Resumen.

Pese a que la necesidad del agua para la fecundación restringe la distribución de los pteridófitos, algunas especies son capaces de vivir en situaciones de notable sequedad. La fecundación en el ciclo sexual puede, por otra parte, estar limitada por otros factores, entre ellos: errores en la fecundación, competencia entre embriones, autoincompatibilidad, acumulación de factores letales esporofíticos (carga genética), y, en algunas especies, posibles interferencias entre entre la expresión de la masculinidad y femineidad en el mismo gametófito.

Los ciclos vitales aberrantes son frecuentes en los helechos europeos. Todos, por lo que se conoce hasta este momento, dependen de la formación de nucleos de restitución, que dan lugar a esporangios con ocho células madres de las esporas. Los embriones son formados apogámicamente. Unos pocos ciclos vitales presentan aposporia y apogamia (y quizas partenogénesis), pero estos casos únicamente se mantienen en cultivo.

Los ciclos heterosporicos también se encuentran en los pteridófitos europeos. En *Marsilea* y *Pilularia* la formación de megásporas viables implica la reabsorción regular de tres esporas en cada tétrada,

de forma similar a lo que ocurre en los espermatófitos. En *Selaginella* la megasporogénesis es menos regular, presentándose la pérdida tanto de las células madre de las esporas y como los productos de la meiosis. La formación de megásporas en *Isoetes* parece seguir un proceso simple a partir de un número menor de células madres en el esporangio. Tanto Selaginella como Isoetes parecen representar intentos dirigidos hacia ciclos heterospóricos de un éxito evolutivo limitado.

Palabras clave: Fecundación, Incompatibilidad, Apogamia, Heterosporia.

INTRODUCTION.

The life cycle of the pteridophyte is one of the best known examples of the "Alternation of Generations". The two generations (sporophyte and gametophyte) are, except for the sporophyte in its embryonic phase, entirely independent of each other. The regular cycle involves fusion of spermatozoid and egg. This is one of the reasons why many pteridophytes flourish where moisture promotes the opening of antheridia and archegonia, and the spermatozoids are able to swim to the egg. Some pteridophytes with normal cycles are nevertheless able to persist in remarkably dry situations. Their reproduction is then dependent on seasonal rainfall or heavy dew. A familiar example is the ubiquitous fern *Anogramma leptophylla*. The sporophyte here is unusual in being ephemeral. The gametophyte, which is partly tuberous and subterranean, is able to withstand the dryness of summer. Fertilization depends upon the moisture of autumn and winter.

FEATURES OF SEXUAL CYCLES.

Examination of gametophytes of the fern *Pteridium* after they have placed in a suspension of spermatozoids shows that many more eggs have been fertilized than yield embryos. The reason for the failure of many zygotes to develop is not known. It may be that the period of viability of the egg cell is very short, and only one, or very few, are capable of further growth at the time of fertilization. Another possible cause may lie in errors of fertilization. If the spermatozoid penetrates the egg obliquely, the sperm nucleus may pass to one side of the egg nucleus instead of directly into it (BELL, 1979). The two nuclei then seem to lie quietly side by side with no attempt at fusion. Finally, competition or hormonal interaction between zygotes may cause a number to regress. Evidence that influences of this kind exist is provided by experiments with dimethyl sulphoxide (DMSO). The presence of a low concentration of DMSO in the medium does not interfere with the growth of the gametophytes of *Pteridium*. After flooding with a suspension of spermatozoids such gametophytes yield, instead of one or two, up to five vigorous sporophytes (SHEFFIELD, 1984). DMSO is known to affect membrane permeability and it may produce its effect by limiting inter-zygotic interaction.

An interesting aspect of the normal homosporous life-cycle is the possibility of self-incompatibility tending to restrict intragametophytic fertilization. The first investigation of this question was undertaken by CZAJA (1921). He concluded that both self- and cross-fertilization were frequent in the species he studied (all non-European ferns). The first European fern to be subjected to careful experimentation was *Pteridium*. WILKIE (1956) investigated three Scottish populations of bracken, gathering his spores from a single frond in each instance. In a preliminary experiment he isolated gametophytes and grew them to sexual maturity. He then compared the effects of flooding one series of isolated gametophytes with water, and a second series with mixed

spermatozoids from the original dense culture. With gametophytes from one population, flooding with water yielded embryos in only 15% of the cultures, whereas spermatozoids were effective in 83%. The results from other populations were equally impressive. In further experiments pairs of gametophytes, randomly selected, were isolated in petri dishes, and when sexually mature flooded with water. In about half the pairs (50-63%) each gametophyte produced a sporophyte. The remainder were either barren (30-50%), or in a few instances (0-9%) only one gametophyte produced a sporophyte. Further experiments with clones raised from the original gametophytes confirmed these results. The indications are therefore that WILKIE's population of *Pteridium* possessed a 1-gene, 2-allele, incompatibility system which severely restricted, but did not entirely eliminated the possibility of self-fertilization. Subsequently KLEKOWSKI (1972) obtained similar results from a population of *Pteridium* in Australia, but results from elsewhere were variable, ranging from indications of a 2-gene incompatibility system to complete self-fertility. In view of these wide divergences KLEKOWSKI prefers to hold recessive sporophytic lethals responsible for infertility in *Pteridium*, and not genetic self-incompatibility. More recently SCHNELLER (1988), working with *Athyrium filix-femina*, provided evidence for the inadequacy of both self-incompatibility and genetic load, acting either separately or together, to explain completely the results of breeding experiments. He concludes that other factors, as yet unidentified, may play a role in determinating the fertility. The extent to which the results obtained with *Pteridium* and *Athyrium* relate to other European ferns is not known.

A possibility not taken into consideration by WILKIE and KLEKOWSKI is that in *Pteridium* oogenesis may not be successfully completed if antheridia are differentiating on the same gametophyte. The gametophytes are initially male. Some (the "ameristic" gametophytes) remain male indefinitely, but the remainder become cordate and begin to bear archegonia. Antheridia, if any, are then confined to basal proliferations. Renewed production of antheridia on the main prothallus is usually associated with cessation of activity of the apical meristem. The apical notch disappears and the production of archegonia ceases. Sporophytes emerging from late-formed archegonia in gametophytes in this condition are often ill-formed. It seems likely that both the production of viable egg cells in *Pteridium* and normal embryogeny depend upon the gametophyte having an active apical meristem. It is not clear whether WILKIE and KLEKOWSKI in setting up their experiments were always dealing with gametophytes which were reproductively normal. A similar uncertainty applies to SCHNELLER's experiments, but oogenesis in *Athyrium* has not been investigated as extensively as in *Pteridium*. In some tropical ferns (e.g. *Lygodium*) antheridia and archegonia are regularly intermixed but so far as known this is not a feature of any European species.

Despite these observations, the evidence from the electrophoresis of extractable proteins is that the reproduction of *Pteridium* is predominantly by outcrossing (WOLF et al., 1987). The explanation may however lie in the temporal separation of antheridia and archegonia in the development of the gametophyte. In European ferns generally *Pteridium* is probably not unique in showing individual variations in the development of the gametophytes. Although most of the gametophytes in a culture of a given species may show one of the sequences of sex-expression recognized by KLEKOWSKI (1969) and MASUYAMA (1975), it is likely that there would always be sufficient deviation to provide for cross-fertilization. Selfing would seem to be improbable in natural conditions without invoking self-incompatibility.

Numerous hybrids between species, and even some between genera (for example, between *Asplenium* and *Phyllitis*) are known in ferns. Nevertheless in many instances hybridity seems to be prevented by the foreign spermatozoid reaching, but failing to penetrate, the egg cell (HOYT, 1910). In some instances in attempting to cross genera (for example *Athyrium* and *Dryopteris*) SCHNELLER (1981) has found that the foreign spermatozoids, although chemotactically attracted to the opened archegonium, are immobilized as they enter the mucilage emerging from the canal. In consequence none reaches the egg cell. The behaviour of spermatozoids in the archegonial mucilage is however complex. In *Pteridium* SHEFFIELD (personal communication) has found that even native spermatozoids are immobilized in the mucilage which first issues from the canal. Only those which arrive later as the first mucilage disperses find a free passage to the egg cell.

ABERRANT LIFE CYCLES.

Some European ferns maintain themselves by aberrant cycles. Particularly notable are *Dryopteris affinis* subsp. *affinis* and associated forms formerly placed under *D. borreri*. The cycle of these ferns, first worked out by the German pteridologist DÖPP (1932; 1939), involves an imperfect mitosis in the final division of the sporogenous lineage. In this mitosis telophase is omitted. Consequently when the nuclear envelope reforms it encloses a doubled number of chromosomes, forming a so-called *restitution nucleus*. Interestingly the cell makes an attempt to divide, but the transverse wall which grows in from the margins of the cell, and which may even pinch the nucleus, is rapidly resorbed (SHEFFIELD et al., 1983). The consequence is that a large spore mother cell is formed, 8 taking up the space which would be occupied by 16 in a normal sexual fern. The spores are correspondingly larger: those of *D. affinis* subsp. *affinis*, for example, may reach a diameter of about 80 µm, compared with about 40 µm in *D. oreades*. Meiosis in *D. affinis* subsp. *affinis*, which has two sets of chromosomes in its nucleus before restitution, is fairly regular. Other apogamous forms related to *D. affinis* have three, four or five sets of chromosomes, and the events in the sporangium are correspondingly less regular. As well as those containing 8 spore mother cells, other sporangia contain 4 or 16. These are mostly barren. An occasional viable spore may be produced. This aspect has not been studied in detail, but persistence might lead to valuable information about the origin of the apogamous species (MANTON, 1950).

The spores of *D. affinis* subsp. *affinis* germinate freely. They pass through a brief male phase, in which perfect spermatozoids are formed, and then become cordate. The apogamous embryo arises in the position in which archegonia would appear in a sexual fern. Subspecies of *D. affinis* containing more than two sets of chromosomes may have arisen by archegonia of a related sexual species having been fertilized by spermatozoids from *D. affinis* subsp. *affinis*. DÖPP (1941) carried out such a hybridization with *D. filix-mas* and found that the progeny was also apogamous. Gametophytes of *D. affinis* subsp. *affinis* and of other apogamous ferns occasionally produce archegonia (LAIRD & SHEFFIELD, 1986). There is no evidence that they are functional, but they have been little studied.

Another form of apogamous cycle, detected in the *Hymenophyllaceae, Polypodiaceae* and *Aspleniaceae*, involves the formation of 32 diplospores in place of 64 normal spores (WALKER, 1985). The nucleus of the spore mother cell enters meiosis, but synapsis is entirely absent. Only the second division of meiosis takes place. In consequence the diplospores contain an unreduced chromosome number. So far as is known this form of cycle is not represented in Europe, but it may yet be found.

A third form of cycle involving apogamy is found in Europe, but only in varieties maintained in cultivation. An example is provided by *Athyrium filix-femina* var. *clarissima*. The fertile frond fails to produce perfect sporangia, but gametophytic outgrowths occur in the soral region. If the frond is in contact with soil they become established and provided a means of propagation. New sporophytes arises either directly from the gametophyte, or (in one form) parthenogenetically from egg cells (FARMER & DIGBY, 1907). Unfortunately this variety is now very rare and it has not been possible to re-investigate it by current techniques.

Apart from the cycles described in the foregoing, aberrations of the normal cycle are not uncommon in experimental conditions. Fronds, or portions of fronds, for example, particularly those that are first formed, will produce gametophytes if detached and laid on soil, or on a mineral-agar medium in sterile conditions (SHEFFIELD & BELL, 1981). Sometimes apospory will occur if the frond is still attached, especially if the petiole is damaged (SHEFFIELD et al., 1982). Aposporous gametophytes are perfect in function. The chromosome number is unreduced, and the gametes can be used to generate triploid or tetraploid sporophytes. It is possible that this has occurred occasionally in Nature, leading to the production of auto- and allotetraploids. Alternatively tetraploids may have been arisen by the chance production of unreduced spores (GASTONY, 1986) or by chance somatic doubling. The latter has been detected in the fronds of haploid plants of

Athyrium filix-femina (SCHNELLER, 1983), and in hybrids between *A. filix-femina* and *A. distensifolium* (SCHNELLER & RASBACH, 1984).

In soil cultures the apogamous development of sporelings from the gametophytes of sexual ferns is usually induced by prolonged culture with watering solely from below so that no gametes are released. By careful control of hydratation it is sometimes possible to obtain normal and apogamous sporelings from the same gametophyte, as, for example, with the diploid gametophytes of *Asplenium septentrionale* (D. E. MEYER, 1952).

If such apogamous sporelings can be brought to maturity an analysis of the behaviour of the chromosomes at meiosis is likely yield insight into the origin of the tetraploid forms. MANTON & WALKER (1954), for example, found only univalents in the prophase of meiosis in apogamous sporelings of *Dryopteris dilatata* and *D. filix-mas*, confirming the allotetraploid nature of these species. Results with supposed autotetraploids have been particularly interesting. Species such as the tetraploid *Asplenium trichomanes* subsp. *quadrivalens* show only bivalents at normal meiosis and behave as diploids (LOVIS, 1977). However, although apogamous sporelings of this subspecies also show bivalents at meiosis, they tend to fall apart at the end of prophase (BOUHARMONT, 1972). This, together with the absence of quadrivalents in normal meiosis, suggest imperfect homology. Tetraploid forms such as *A. trichomanes* subsp. *quadrivalens* may have arisen by chromosome doubling of hybrids between slightly different genotypes, possibly formed at sites of overlap of different ecotypes. Perfect autotetraploids raised from aposporously generated gametophytes show quadrivalents at meiosis (VIDA, 1970) and their growth (as in *Pteridium*, K. I. MEYER, 1945; personal observations) is often irregular. It seems unlikely that such forms would become established in the wild. The mechanism by which a perfect autotetraploid could become "diploidized" is also unknown.

Present evidence suggest that the successful production of apogamous forms by ferns with a sexual cycle depends upon the gametophytic nucleus being at least diploid and possibly hybrid. Certainly in a species such as *Pteridium*, which behaves genetically as a diploid (WOLF et al., 1987), apogamy can be achieved only with difficulty (WHITTIER & STEEVES, 1960) and the outgrowths fail to become established. *Phyllitis scolopendrium* provides an apparent exception and deserves further study. Although believed to be a simple diploid (MANTON, 1950), apogamous sporeling can be obtained relatively easily and even occur in Nature (REEKMANS, 1959). The readiness with which *Phyllitis* throws crested varieties (ANDERSSON-KOTTÖ, 1929) and forms with abnormal reproduction (ANDERSSON-KOTTÖ & GAIRDNER, 1936) may indicate some kind of genetic instability which also affects the normal cycle.

In the homosporous fern allies the life cycles, so far as known, are sexual. Apogamy may occur in cultures, but the cytological relationships are not clear.

HETEROSPORUS CYCLES.

Pilularia and *Marsilea* display true heterospory. The developing megasporangium in these ferns contains several megaspore mother cells. Each undergoes meiosis, and in the electron microscope the four megaspores in each newly-formed tetrad are visually identical (Fig. 1). Nevertheless, selective degeneration begins in the tetrad (Fig. 2) and continues as the tetrad opens. Only one spore in each tetrad survives. Competition between the surviving megaspores reduces their number still further. When the megasporangium is ripe, it normally contains only one large megaspore filled with food reserves (BELL, 1985). Regular resorption of three of the meiotic products in each tetrad seems to represent a distinct jump in evolution. It is not a feature likely to have been arrived at progressively from simple inequalities in spore size. The same kind of heterospory is found in the seed plants. Some of the earliest seed plants, like *Marsilea* but unlike the living forms, also produced their megaspores in tetrahedral tetrads (PETTITT, 1969).

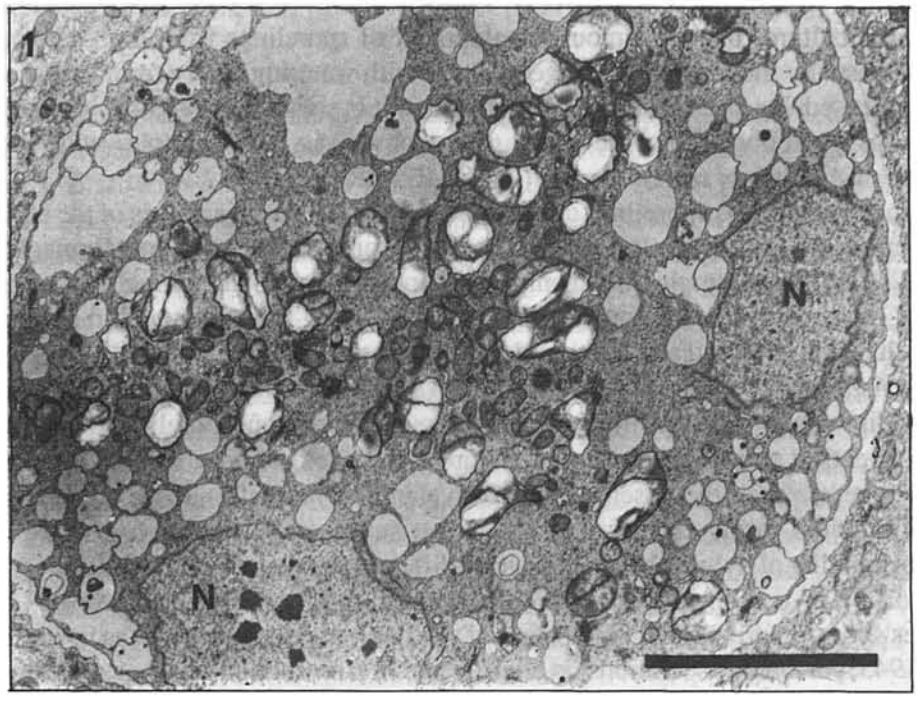

Fig. 1. *Marsilea vestita*. Section of a very young tetrad of megaspores before the formation of dividing walls. Two nuclei (N) are visible. The mitochondria and plastids (which contain starch and which appear white) are congregated at the cleavage planes (three of which are sectioned). No differences are evident between the spores. Scale 5 µm.

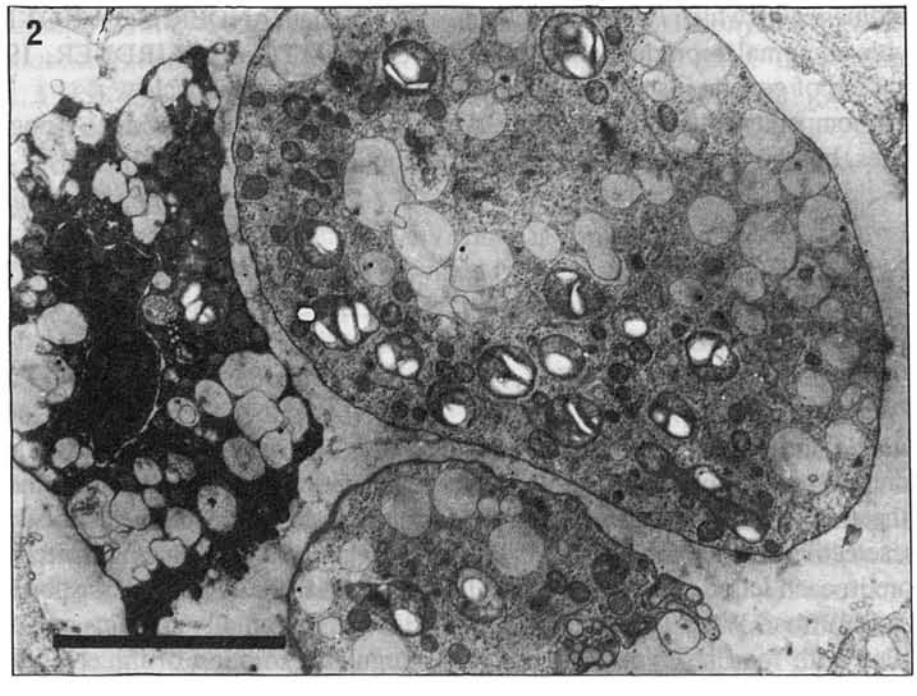

Fig. 2. *Marsilea vestita*. Section of a later tetrad. One megaspore (left) has begun to degenerate before the spores of the tetrad separate. Scale 5 µm.

Parthenogenesis occurs in *Marsilea* and *Pilularia* in experimental conditions (NATHANSON, 1900), but it is not known whether it is a feature of European species in Nature. It is however found in species of *Marsilea* frequenting seasonal pools in North India (BHARDWAJA & ABDULLAH, 1972; BHARDWAJA, personal communication). The cytology has still to be worked out.

Megasporogenesis in *Selaginella* is less regular than in *Marsilea*. Some of the megaspore mother cells fail to complete meiosis, and not all the megaspores reach maturity (PETTITT, 1971, 1977). In *Isoetes* megasporangia and microsporangia differ sharply in the number of spore mother cells, and there is no ordered degeneration of meiotic products on the female side (although there may be resorption of archaesporial tissue earlier in the female lineage). Both *Selaginella* and *Isoetes* seem to be experiments in alternative forms of heterospory of limited evolutionary success.

CONCLUSIONS.

The life cycles of the European pteridophytes provide a wealth of material for experimental investigation both in the laboratory and in the field. It is not known, for example, whether antheridiogens, which are produced and liberated by young gametophytes of ferns, and which tend to hold *in vitro* cultures in the male phase. (NÄF, 1979), are equally effective in the field. In natural conditions they may be rapidly destroyed by soil organisms. The origins and genomic constitutions of the apogamous ferns such as *Dryopteris affinis* and *Pteris cretica* have still to be firmly established. Although the apogamous ferns are generally thought to be of hybrid origin (MANTON, 1950), this has been questioned (GASTONY & GOTTLIEB, 1985; GASTONY, 1985). Electrophoretic evidence indicates that apogamous forms of *Pellaea* native to the United States may have had an autoploid origin. Study of the Pteridophyta can also contribute significantly to solving the problems of the Alternation of Generations. Advances in electron microscopy such as immunogold cytochemistry applied to sporogenesis and gametogenesis, for example, will begin to reveal why spores grow into gametophytes and zygotes into sporophytes.

All the materials necessary for research into these fundamental areas of plant biology are available to the European pteridologist.

REFERENCES

ANDERSSON-KOTTÖ, I. (1929). A genetical investigation in Scolopendrium vulgare. *Hereditas*, 12: 109-178.

ANDERSSON-KOTTÖ, I. & GAIRDNER, A.E. (1936). Inheritance of apospory in Scolopendrium vulgare. *J. Gen.*, 32: 189-228.

BELL, P.R. (1979). Gametogenesis and fertilization. In A.F. Dyer (ed.), *The experimental biology of ferns*, pp. 471-503. Academic Press, London.

BELL, P.R. (1985). Maturation of the megaspore in Marsilea vestita. *Proc. r. Soc. London*, B223: 485-494.

BHARDWAJA, T.N. & ABDULLAH, S. (1972). Some observations on parthenogenetic sporelings of the water fern Marsilea. *Nova Hedwigia*, 21: 521-528.

BOUHARMONT, J. (1972). Meiosis and fertility in apogamously produced diploid plants of Asplenium trichomanes. In C.D. Darlington and K.R. Lewis (eds.), *Chromosomes today*, vol. 3, pp. 253-258, Longman, London.

CZAJA, A.Th. (1921). Über Befruchtung, Bastardierung und Geschlechtertrennung bei Prothallien homosporer Farne. *Z. Bot.*, 13: 545-589.
DÖPP, W. (1932). Die Apogamie bei Aspidium remotum. *Al. Br. Planta*, 17: 86-152.
DÖPP, W. (1939). Cytologische und genetische Untersuchungen innerhalb der Gattung Dryopteris. *Planta*, 29: 481-533.
DÖPP, W. (1941). Über Dryopteris paleacea Christensen. *Ber. dtsch. bot. Ges.*, 59: 423-426.
GASTONY, G.J. (1985). The origins of apogamous ferns. *Amer. J. Bot.*, 72: 917-918.
GASTONY, G.J. (1986). Electrophoretic evidence for the origin of fern species by unreduced spores. *Amer. J. Bot.*, 73: 1563-1569.
GASTONY, G.J. & GOTTLIEB, L.D. (1985). Genetic variation in the homosporous fern Pellaea andromedifolia. *Amer. J. Bot.*, 72: 257-267.
HOYT, W.D. (1910). Physiological aspects of fertilization and hybridization in ferns. *Bot. Gaz.*, 49: 340-370.
KLEKOWSKI, E.J. Jr. (1969). Reproductive biology of the Pteridophyta. II. Theoretical considerations. *Bot. J. Linn. Soc.*, 62: 347-359.
KLEKOWSKI, E.J. Jr. (1972). Evidence against genetic self-incompatibility in the homosporous fern Pteridium aquilinum. *Evolution*, 26: 66-73.
LAIRD, S. & SHEFFIELD, E. (1986). Antheridia and archegonia of the apogamous fern Pteris cretica. *Ann. Bot.*, 57: 139-143.
LOVIS, J.D. (1977). Evolutionary patterns and processes in ferns. *Adv. Bot. Res.*, 4: 230-415.
MANTON, I. (1950). *Problems of cytology and evolution in the Pteridophyta.* University Press, Cambridge.
MANTON, I. & WALKER, S. (1954). Induced apogamy in Dryopteris dilatata (Hoffm.) A. Gray and D. filix-mas (L.) Schott emend. and its significance for the interpretation of the two species. *Ann. Bot.*, 18: 377-383.
MASUYAMA, S. (1975). The sequence of the gametangium formation in homosporous fern gametophytes. Sci. Rep. *Kyoiku Daigaku Tokyo Sec.*, B 16: 47-69.
MEYER, D.E. (1952). Untersuchungen über Bastardierung in der Gattung Asplenium. *Bibliotheca Bot.*, 123: 1-33.
MEYER, K.I. (1945). Apospory and polyploid forms in Pteridium aquilinum (L.) Kuhn. *Bull. Soc. Nat. Moscow, Sect. Biology*, 50: 74-87.
NÄF, U. (1979). Antheridiogens and antheridial development. In A.F. Dyer (ed.),*The experimental biology of ferns*, pp. 436-470. Academic Press, London.
NATHANSON, A. (1900). Ueber Parthenogenesis bei Marsilia und ihre Abhängigkeit von der Temperatur. *Ber. dtsch. bot. Ges.*, 18: 99-109.
PETTITT, J.M. (1969). Pteridophytic features in some Lower Carboniferous seed megaspores. *Bot. J. Linn. Soc.*, 62: 233-239.
PETTITT, J.M. (1971). Developmental mechanisms in heterospory. I. Megasporocyte degeneration in Selaginella. *Bot. J. Linn. Soc.*, 64: 237-246.
PETTITT, J.M. (1977). Developmental mechanisms in heterospory: Features of post-meiotic regression in Selaginella. *Ann. Bot.*, 41: 117-125.
REEKMANS, M. (1959). Contribution à l'étude de l'apogamie chez Asplenium scolopendrium L. *Lejeunia*, 21: 21-28.
SCHNELLER, J.J. (1981). Evidence for intergeneric incompatibility in ferns. *Pl. Syst. Evol.*, 137: 45-56.
SCHNELLER, J.J. (1983). Haploid sporophytes in *Athyrium filix-femina* (L.). Roth and evidence for somatic diploidization in one of them. *Bot. Helvetica*, 93: 85-90.
SCHNELLER, J.J. (1988). Remarks on reproductive biology of homosporous ferns. *Pl. Syst. Evol.*, 161: 91-94.
SCHENELLER, J.J. & RASBACH, H. (1984). Hybrids and polyploidy in the genus Athyrium (Pteridophyta) in Europe. *Bot. Helvetica*, 94: 81-89.
SHEFFIELD, E. (1984). Effects of dimethyl sulphoxide on the gametophytic and sporophytic phases of Pteridium aquilinum (L.) Kuhn. *Ann. Bot.*, 54: 531-536.

SHEFFIELD, E. & BELL, P.R. (1981). Experimental studies of apospory in ferns. *Ann. Bot.*, 47: 187-195.
SHEFFIELD, E., BELL, P.R. & LAIRD, S. (1982). Tracheary occlusions in fronds of Pteridium aquilinum (L.) Kuhn showing apospory. *New Phytol.*, 90: 321-325.
SHEFFIELD, E., LAIRD, S. & BELL, P.R. (1983). Ultrastructural aspects of sporogenesis in the apogamous fern Dryopteris borreri. *J. Cell. Sci.*, 63: 125-134.
VIDA, G. (1970). The nature of polyploidy in Asplenium rutamuraria L. and A. lepidum C. Presl. *Caryologia*, 23: 525-547.
WALKER, T.G. (1985): Some aspects of agamospory in ferns- the Braithwaite system. *Proc. r. Soc. Edin.*, 86B: 59-66.
WHITTIER, D.P. & STEEVES, T.A. (1960). The induction of apogamy in the bracken fern. *Canad. J. Bot.*, 38: 925-930.
WILKIE, D. (1956). Incompatibility in bracken. *Heredity*, 10: 247-256.
WOLF, P.G., HAUFLER, C.H. & SHEFFIELD, E. (1987). Electrophoretic evidence for genetic diploidy in the bracken fern (Pteridium aquilinum). *Science*, 236: 947-949.